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(57) Abstract

The present invention relates to various strains of *S. aureus* BS449, strains of *S. aureus* ZO3984 and strains of *S. aureus* VLVL8407 and combinations thereof. The above strains were deposited and registered on the 16th December, 1997 in the CNM Collection de Cultures de Microorganismes de l'Institut Pasteur. The invention also relates to a vaccine against the occurrence of bovine mastitis comprising a combination of the above strains of *"S. aureus"* and to the vaccination of cows against the occurrence of bovine mastitis infection with said vaccine.

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ISOLATED STRAINS OF *Staphylococcus aureus* AND VACCINES MANUFACTURED THEREFROM

The present invention relates to isolated strains of *Staphylococcus aureus* and vaccines manufactured from same against udder infection caused by *Staphylococcus aureus*.

Bovine mastitis is the most important infectious disease affecting both the quality and quantity of milk production. *Staphylococcus aureus* (hereinafter defined: *"S. aureus"*) is the prime agent causing bovine mastitis and it is difficult to eliminate. In different countries the prevalence of *S. aureus* mastitis ranges from 10% to 40% of all cows. The infected animals may serve as reservoirs of infection endangering other dairy cattle in the herd (Fox, L.K. and Hancock, D. 1989. Effects of segregation on prevention of intramammary infection by *Staphylococcus aureus* J. Dairy Sci. 72:540-544).

Recent estimates suggest that the annual production losses due to *S. aureus* are over 15 million dollars in Israel and over 2 billion dollars in the USA. The prevalence of *S.aureus* mastitis in dairy cattle raises several concerns. This bacterium can cause severe damage to milk-synthesizing tissues, drastically reducing milk production and altering milk composition (See: (1) Oliver, S.P., Sordillo, L.M. 1988. Udder health in the periparturient period. J Dairy Sci. 71:2584-2606; (2) Postle, D.S., Roguinsky, M., Poutrel, B. 1978. Induced Staphylococcal infections in the bovine mammary gland. Am J Vet Res.39:29-35; (3) Sordillo, L.M., Nickerson, S.C and Akers, R.M. 1989. Pathology of mastitis during lactogenesis: Relationships with bovine mammary structure and function. J. Dairy Sci. 72: 228-240; (4) Watson, D.L., McColl, M.L., Davies, H.I. 1996. Field trial of a Staphylococcal mastitis vaccine in dairy herds: clinical, subclinical and microbiological assessments. Aust. Vet. J. 74:447-450.)

Depending on the duration and the severity of the infection, the productive performance of dairy cattle may be diminished permanently.

Developing effective methods of controlling *S. aureus*

mastitis will increase profitability to dairy producers by reducing costs. So far post-milking teat disinfection and antibiotic therapy are the only widely accepted methods of mastitis control (National Mastitis Council. 1987. Current concepts of bovine mastitis. Arlington VA)

These methods are not cost-effective due to milk loss during and after antibiotic therapy. Moreover, antibiotic therapies formulated for intramammary use are generally unsuccessful in eliminating existing *S. aureus* infections or preventing the establishment of chronic diseases (Ziv, G. 1995. Treatment of Mastitis: An overview of progress during the last ten years. Proc The 3rd Internal Mastitis Seminar. Tel Aviv, Israel 2-12).

There is also a growing concern over the presence of drug residues in the food supply as a consequence of these procedures. To date, culling chronically infected cows is often the only practical means of eliminating *S. aureus* from a herd.

Vaccination is a logical approach for controlling infectious diseases in food producing animals. However, the paucity of information regarding relevant antigens remains a major deterrent to successful immunization against *S. aureus* mastitis.

To our knowledge the known commercially available *S. aureus* vaccines have shown limited efficacy under field conditions, e.g.:

In the USA:

1) Nickerson, S.C., Owens, W.E., Bodie, R.L 1993. Effect of a *Staphylococcus aureus* bacterin on serum antibody, new infection, and mammary histology in non lactating dairy cows. J. Dairy Sci. 76:1290-1297;
2) Sears, P.M., Norcross. N.L., Kenny, K., Smith, B., Gonzalez, R.N., Romano, M.N. 1990. Resistance to *Staphylococcus aureus* infections in staphylococcal vaccinated heifers. Proc. Internatl. Symp. Bovine Mastitis. Indianapolis, IN., pp 69.
3) Sordillo, L.M., Nickerson, S.C and Akers, R.M. 1989. Pathology of mastitis during lactogenesis: Relationships with bovine mammary structure and function. J. Dairy Sci. 72: 228-240; and
4) Yoshida K., Ichiman, Y., Narikawa, S., Evans, G.B. 1984. Staphylococcal capsular for preventing mastitis in two herds

in Georgia. J. Dairy Sci. 67:620-627.

In Australia:

1) Watson, D.L. 1984. Evaluation of attenuated, live staphylococcal mastitis vaccine in lactating heifers. J. Dairy Sci. 67:2608-2613;

2) Watson, D.L., Schwartzkoff, C.L. 1990. A field trial to test the efficacy of a staphylococcal mastitis vaccine in commercial dairies in Australia. International Symposium on Bovine Mastitis, National Mastitis Council, Arlington, 73-76;

3) Watson, D.L., 1992. Vaccination against experimental staphylococcal mastitis in dairy heifers. Res. Vet. Sci. 53:346-353; and

4) Watson, D.L., McColl, M.L., Davies, H.I. 1996. Field trial of a Staphylococcal mastitis vaccine in dairy herds: clinical, subclinical and microbiological assessments. Aust. Vet. J. 74:447-450.

In Norway:

1) Nordhaug, M.L., Nesse, L. L., Norcross, N.L., Gudding, R. 1994. A field trial with an experimental vaccine against *Staphylococcus aureus* mastitis in cattle. I. Clinical parameters. J Dairy Sci. 77:1267-1275;

2) Pankey, J.W. et al. 1985. Evaluation of protein A and a commercial bacterin as vaccines against *Staphylococcus aureus* mastitis by experimental challenge. J Dairy Sci. 68:726-731; and

3) Yoshida K., Ichiman, Y., Narikawa, S., Evans, G.B. 1984. Staphylococcal capsular for preventing mastitis in two herds in Georgia. J Dairy Sci. 67:620-627.

For the most part, these conventional vaccines have not prevented the disease and show only a marginal benefit in ameliorating the severity and duration of clinical symptoms of *S. aureus* mastitis. Traditional *S. aureus* mastitis vaccines have included killed or attenuated bacteria, toxoids, and cell wall extracts from selected laboratory or field strains. (See: (1) Nickerson, S.C.; (2) Sears, P.M.; (3) Watson, D.L. 1984; and (4) Watson, D.L., 1992; mentioned above.

These previous attempts have not considered the significant

variation among the different strains of *S.aureus* causing mastitis.

Attempts to solve this problem are described in U.S. Patent Specification No. 4,840, 794. However this solution has not been satisfactory.

It has thus been desirable to find a vaccine which would overcome the above disadvantages, i.e. should-prevent the occurrence of bovine mastitis infection or at least enable the control thereof to a large extent.

It has been shown that such vaccine cannot be prepared from known strains of *S.aureus* and therefore new strains had to be isolated.

The present invention thus consists in the following three field strains of *S. aureus* either separately or together:

strain BS449;

strain Zo3984; and

strain VLVL8407.

Said strains are characterized by the following basic features:

strain BS449: ß-hemolytic; coagulase positive; and phage type 81;

strain ZO3984: ß-hemolytic; coagulase positive; and phage type 3/A, 3/C, 55, and 71; and

strain VLVL8407: unhemolytic; coagulase positive; and phage untypable.

Further biochemical and enzymatic features are shown in Table I hereinafter.

The above strains have been deposited and registered on the 16th December, 1997, in the CNCM Collection Nationale de Cultures de Microorganismes Institut Pasteur under Identification Reference BS 449 (449) I - 1950; ZO 3984 (84) I - 1951; and VLVL 8407 I - 1952

The deposit represents a biologically pure culture of each deposited strain. The deposit is available as required by foreign patent laws in countries were counterparts of the subject application or its progeny are filed and under the conditions of the Budapest Treaty. However, it should be understood that the availability of said deposit does not constitute a license to

practice the subject invention in derogation of the patent rights granted.

Said strains are isolated from cows known as chronically infected and showing a high variability in their electrophoretic profiles and were chosen from four-hundred isolations.

The group of strains was isolated as follows:

Duplicate quarter foremilk samples obtained as described above were taken aseptically according to "International Dairy Federation, Laboratory methods for use in mastitis work, Document 132, Brussels, Belgium" and then submitted to the laboratory.

The bacteriological analysis was performed according to the standards of "National Mastitis Council, 1987, Laboratory and Field Hand Book on Bovine Mastitis, W.H. Hoard and Sons Co., Fort Atkinson WI". 0.01 ml from each milk sample was spread over blood-agar plates (Bacto-Agar; Difco Laboratory) containing 5% washed sheep red blood cells. The minimal detection limit was 5 colony-forming units. The Bacteria were classified as *S. aureus* according to their morphology: 1-3 mm in diameter of bacterial colony, circular smooth and raised with a butyrous consistency, type of hemolysis on blood agar, cultivated on selective media (Baird Parker and Toluidin Blue DNase), a coagulase test (in rabbit plasma) (Coagulative enzyme), agglutination (specific antibodies against *S. aureus)* (Remel Santa Fe, K.S U.S.A., phage typing (Blair and Williams 1961, Bull. Wld. HIth. Org. 1961, 24, 771-784) using the international set of typing phages for human strains. The biochemical and enzyme characterization was performed by ID-32 API STAPH (Bio Merieux Vitex, Inc. Mo. U.S.A.). An antibiogram test was also performed

From the four-hundred strains obtained the above strains were isolated as indicated hereinafter.

The Biochemical and Enzymatic characterization are shown in Table I.

Table I

	VLVL8407	ZO3984	BS449
Phage type	-	3/A,3/C, 55,71	81
Hemolysis	-	ß	ß
Coagulase	+	+	+
GLU Glucose	+	+	+
FRU Fructose	+	+	+
MAL Maltose	+	+	+
LAC Lactose	+	+	+
TRE Trehalose	+	+	+
MAN Manitol	+	+	+
RAF Rafinose	+	-	-
RIB Ribose	-	-	-
CEL Celobiose	-	-	-
NIT Nitrates (reduction)	+	+	+
VP (Actoin Production)	+	+	+
ßGal ß Galctosidase	-	-	-
ArgA Arginine Arylamidase	-	-	-
PAL Alkaline Phosphatase	+	+	+
PyrA Pyrrolidonyl Arylamidase	+	+	+
NOVO Novobiocin (Resistance)	-	+	-
Fermentation:			
SAC Sucrose	+	+	+
NAG N-Acetyl-glucosamine	+	+	+
TUR Turanose	+	+	+
ARA Arbinose	-	-	-
ßGUR ß Glucuronidase	-	-	-
URE Urease	+	-	+
ADH Arginin dihydrolase	+	+	+
ODC Ornithine decaboxilase	-	-	-
ESC Esculine (hydrolysis)	-	-	-

The antibiograms are shown in Table II.

Table II

	ZO3984	VLVL8407	BS449
METHICILLIN (N) *	+	+	+
PENICILLIN (P)	-	-	-
OXYTETRACYCLINE (T)	±	-	-
ERYTHROMYCIN (E)	±	+	+
CEPHALOTHIN (CR)	+	+	+
NOVOBIOCIN (NB)	+	+	±
NORFLOXACIN (NEF)	+	+	+
SXT (SXT)	-	-	-

* METHICILLIN represents CLOXACILLIN and NAFCILLIN

Another aspect of the present invention is a vaccine against the occurrence of bovine mastitis infection consisting of a combination of the above three bacterial strains.

In a preferred embodiment the above bacteria combination is admixed with an adjuvant, advantageously with an Incomplete Freund Adjuvant (IFA) (Difco).

In a preferred embodiment the vaccine is a combination of 0.33 ml of each of the above strains with the Incomplete Freund Adjuvant in a ratio of 1:1 to give a final volume of 2 ml.

Said Vaccine upon challenge protects cows from udder disease following intermammary infection with *S. aureus*.

The present invention thus consists also in the vaccination of cows against the occurrence of bovine mastitis infection with a vaccine according to the present invention.

Said vaccination is performed to heifers advantageously 50 to 60 days before the first parturition and then boosted 30 days

thereafter. Cows are advantageously vaccinated 30 days before the second or any consecutive parturition.

The cows are preferably vaccinated subcutaneously (sc), under the tail root and in the area of the supra mammary lymph node.

The present invention will now be illustrated with the following examples and figures without being limited by same:

Example 1

Separation of the *S. aureus* crude extract.

Cultural medium:

Colombia broth (Difco) was modified by the addition of 0.1% d-glucose, 1% yeast extract and 0.5% NaCl. (See Lee, J.C. et al, 1987, Infection and Immunity, pages 2191-2197.) The medium was autoclaved at 115°C for 15 min., then incubated for 24 - 28 hr at 37°C and then checked for the sterility of the culture.

Each of the 3 strains BS449, Z03984 and VLVL8407 was grown for 24 hr at 37°C in a 5-liter Erlenmeyer containing 1 liter of the medium. At the end of the growth period the broth was checked for the purity of the culture.

The bacteria strains BS449 and ZO3984 were harvested by centrifugation (3000 x g, for 15 min. at 4°C)and then washed 3 times in phosphate buffered saline (PBS) at pH 7.2.

The bacteria pellets [3.3 g/L (wet weight)] were each suspended in approximately 500 ml of PBS and subjected to mechanical agitation with glass beads by cell homogenizer (B. Braun Melsungen AG, Germany) for 10-15 minutes. The glass beads and the remaining bacteria were removed by centrifugation and

discarded. The remaining solution was filtered through 0.80 and 0.45 μm-pore-size membranes. The following enzymes were added to the filtrate solution: 250μg/100 ml of DNase and 750μg/100 ml RNase (Worthington Diagnostics, Freehold, N.J.), which was then incubated for 1 hr at 37°C. After incubation 2ml of 0.1mM phenylmethylsulfonyl fluoride (Sigma) in Acetone and 0.2 ml of 10 mM Tosyl (Sigma) were added to a 100 ml solution. Each solution was checked for the sterility of the culture. The protein concentrate in the solution was assayed by Braedford (Bio-Rad, UK) and according to the concentration (±0.3 mg/ml), the solution of each bacteria was aliquoted and kept at -80°C. The bacteria solutions of strains BS449 and ZO3984 were marked (BSs) and (ZOs), respectively.

The culture supernatant of the VLVL8407 strain (marked VLs) was centrifugated (3000 x g for 15 min. at 4°C) and then filtered through 0.45μm-pore-size membranes and finally concentrated to approximately 1:10 from the original volume. The concentration was performed in a cellulose tubular membrane (Nominal MWCO: 3500) (Cellu. Sep. Texas USA) by Polyethylene glycol 35,000 (Fluka, Switzerland) at 4°C. At the end the concentrate of the supernatant (VLs) was dialyzed against PBS (pH 7.2, 4°C, 48 hr). The VLs was checked for the sterility of the culture. The protein concentrate in the VLs was assayed by Braedford (Bio-Rad, UK) and according to the concentrations (±0.3mg/ml) was aliquoted and kept at -80°C.

The antigen analysis of BSs, ZOs and VLs was performed by polyacrylamide gel electrophoresis (PAGE) (Lemlli, U.K. 1970. Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680) for protein and the

glycoprotein profile by Immuno-blot kit (Bio-Rad).

Example 2

Vaccine preparation and vaccination

Before vaccination, BSs, ZOs and VLs were thawed and 0.33ml of each was mixed 1:1 with Incomplete Freund Adjuvant (IFA) (Difco) to give a final volume of 2 ml/cow. Each cow was vaccinated subcutaneously (sc), 1 ml under the tail root and 1 ml in the area of the supra mammary lymph node.

Said vaccination was performed to heifers 50 to 60 days before the first parturition and then boosted 30 days thereafter. Cows were vaccinated 30 days before the second parturition.

Example 3

Toxicity and efficacy of the vaccines

The toxicity of the vaccine (each batch) was tested in a group of 8 mice (BALB/C). Mice were inoculated intraperitoneally (IP) with 1 ml of the BSs, ZOs abd VLs mixed. The mice were under surveillance for 1 week. None of these mice showed any symptom of toxicity, no mortality or morbidity.

The efficacy of the vaccine was tested by vaccination of mice Sc with 0.1 ml of the final vaccine (with IFA). The sera of the mice (prior to and post vaccination) were tested for antibody against the BSs, ZOs and VLs antigens by Westernblot (Towbin, H., Staehelin, T. and Gordon, J. 1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets:

procedure and some application. (Proc. Natl. Acad, Sci. USA. 76:4350).

Some results of mice vaccination with the vaccine are summarized in Fig. 1

Example 4

Two consecutive experiments were conducted, A and B. Each experiment included 10 Israeli Holstein cows in mid-lactation, yielding about 25-35 Kg/day milk free of bacterial infection or contaminated with minor pathogens and containing low Somatic Cell Counts (SCC) ($<300X10^3$/ml). In each experiment, the cows were divided into two groups according to the period of time from the last parturition, milk yield, SCC and the status of the udder contamination. Before vaccination, blood and milk were collected from each cow and were tested for specific antibodies by immunoblot.

The cows in group 1 were vaccinated with the vaccine (BSs, ZOs, VLs mixed 1:1 with IFA). 1 - 1.5 ml of the vaccine was injected subcutaneously (sc) under the tail root and additional 1 - 1.5 ml was administered sc in the area of the supra mammary lymph node, while the cows in group 2 were injected similarly with IFA+PBS. The time of vaccination, boosts, blood collection, bacteriology examination, determination of SCC and antibody in milk or blood are summarized in Fig. 2 for both experiments.

The cows were boosted once in Exp. A, and twice in Exp. B. The cows were challenged with 1000 CFU/quarter with *S. aureus* VLVL8407, each in two quarters. Milk and blood samples were collected during the post challenge period in order to examine

the bacteriologic status, determined by SCC and antibody levels (Fig. 2).

After the termination of the experiments sections of the injection area were submitted to histopathology.

Results

The percentage of the infected quarters and of the cows, two weeks after challenge are summarized in Fig. 3 In Exp. B, one of the vaccinated cow was excluded before challenge because drying off (next parturition).

In Exp. A, 8/10 quarters of the control group and only 3/9 quarters in the vaccinated one were shading *S. aureus*. In the second Exp. 11/11 quarters of the control group shaded *S. aureus*. and only 3/8 in the vaccinated group did so.

Combining the two Exp. 19/21 quarters of the control group shaded *S. aureus* while only 6/17 quarters of the vaccinated cows did so. The difference between vaccinated and control cows according to the number of quarters shading *S. aureus*, was statistically significant ($P = 0.001$).

The results are summarized in Fig. 3.

The SCC, two weeks after challenge, for the quarters that shaded *S. aureus* were 540×10^3 (19/21) in the control group in comparison to 100×10^3 (6/17) in the vaccinated one. This difference is highly statistical significant ($P<0.0001$).

The results are summarized in Fig. 4.

The histopathological examination of the tissues around the injected area revealed normal structure with no pathological finding.

Claims

1. Strain of *S. aureus* BS449.
2. Strain of *S. aureus* ZO3984.
3. Strain of *S. aureus* VLVL8407.
4. A combination of the strains of *S. aureus* claimed in Claims 1 to 3.
5. Strain BS449: ß-hemolytic; coagulase positive; and phage type 81.
6. Strain ZO3984: ß-hemolytic; coagulase positive; and phage type 3/A, 3/C, 55, and 71.
7. Strain VLVL8407: unhemolytic; coagulase positive; and phage untypable.
8. A combination of the strains of *S. aureus* claimed in Claims 5 to 7.
9. Strain BS449 having the features shown in Table I.
10. Strain ZO3984 having the features shown in Table I.
11. Strain VLVL8407 having the features shown in Table I.
12. A combination of the strains of *S. aureus* claimed in Claims 9 to 11.
13. A vaccine against the occurrence of bovine mastitis infection consisting of a combination of the three bacterial strains according to Claim 4, 8 or 12.
14. A vaccine according to Claim 13, in combination with an Adjuvant.
15. A vaccine according to Claim 14, wherein the Adjuvant is Incomplete Freund Adjuvant.
16. A vaccine according to any of Claims 13 to 15, wherein the vaccine is a combination of 0.33 ml of each of the strains of *S. aureus* claimed in any of Claims 1 to 3, 5 to 7 or 9 to

11 with the Incomplete Freund Adjuvant in a ratio of 1:1 giving a final volume of 2 ml.

17 . Vaccination of cows against the occurrence of bovine mastitis infection with a vaccine according to any of Claims 13 to 16.

18. Vaccination according to Claim 17 which is performed by vaccinating the cows subcutaneously (sc), under the tail root and in the area of the supra mammary lymph node.

Immunoblot of mice sera against BSs antigen before and after vaccination with *S. aureus* vaccine.

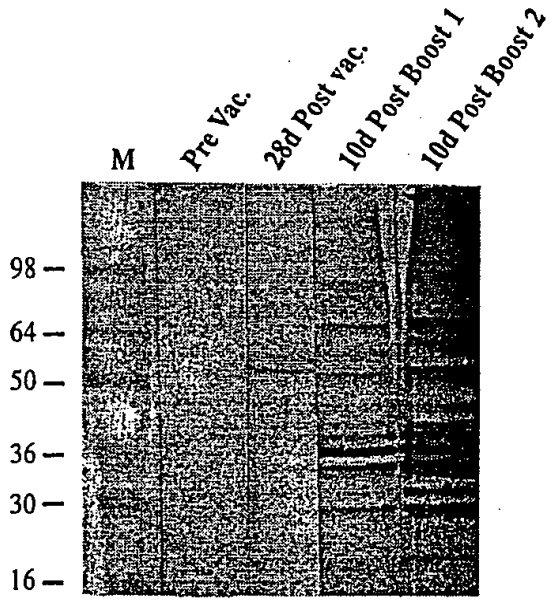


Fig 1

Time table of the two vaccinated and challenged trials in cow's study

Trial 1.

-60	-25	-13	0	13	22	37	43	57	63	65	66	70	73	78	84
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
			Vacc.			Boost		"Volcani"		Challenge					Histology

Trial 2.

-30	-5	0	9	21	36	45	56	69	72	77	78	79	84	91	98
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
		Vacc.			Boost		Boost	"Volcani"		Challenge					Histology

* Test: Bacteriology, SCC, Differentiate count, NAGase, and Antibody in blood and milk

Fig 2

Precentage of infected cows, quarters two weeks after experimental challenge with *S. aureus* previously vaccinated either with S. Vacl or with vehicle only (control)

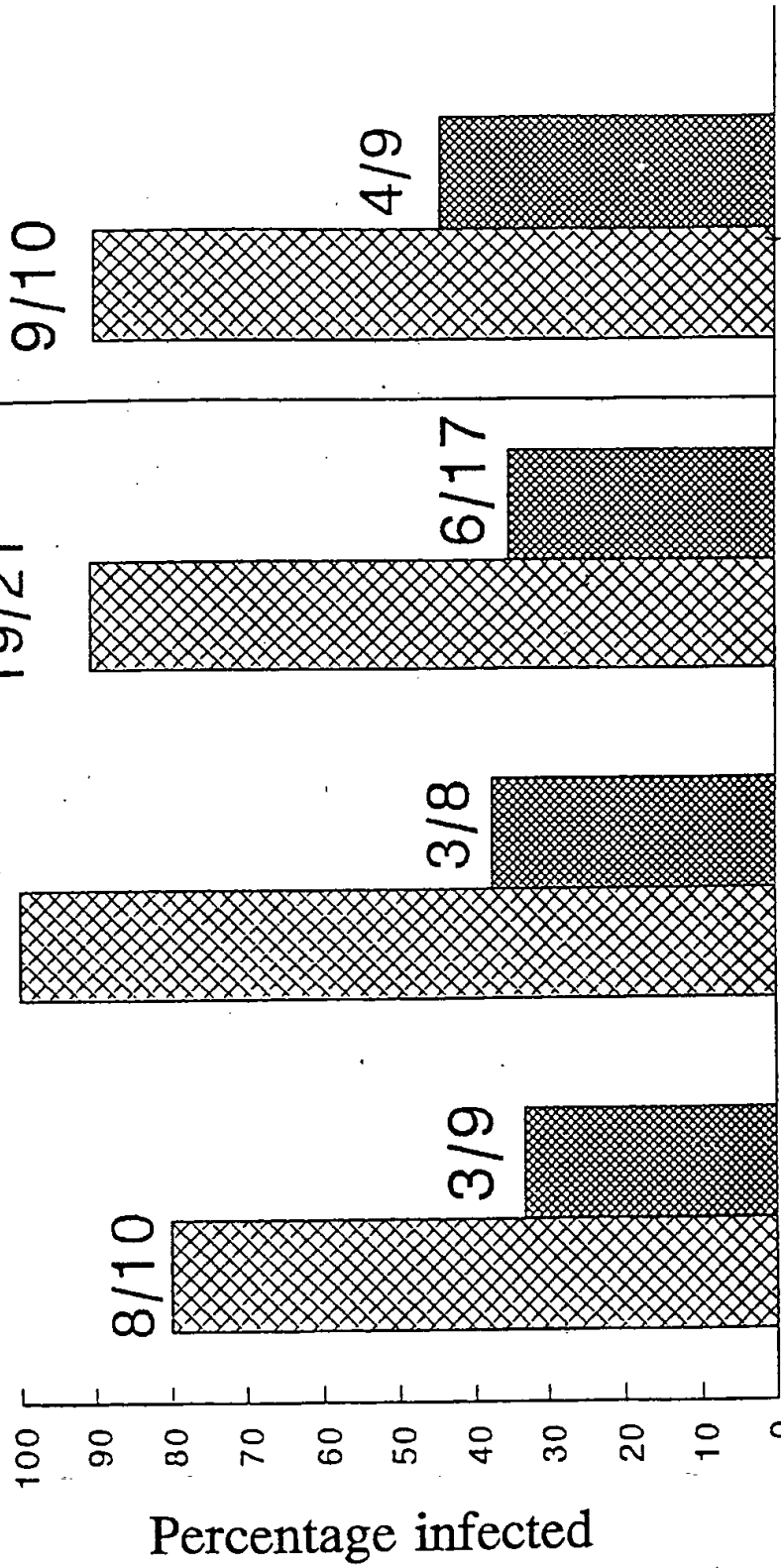
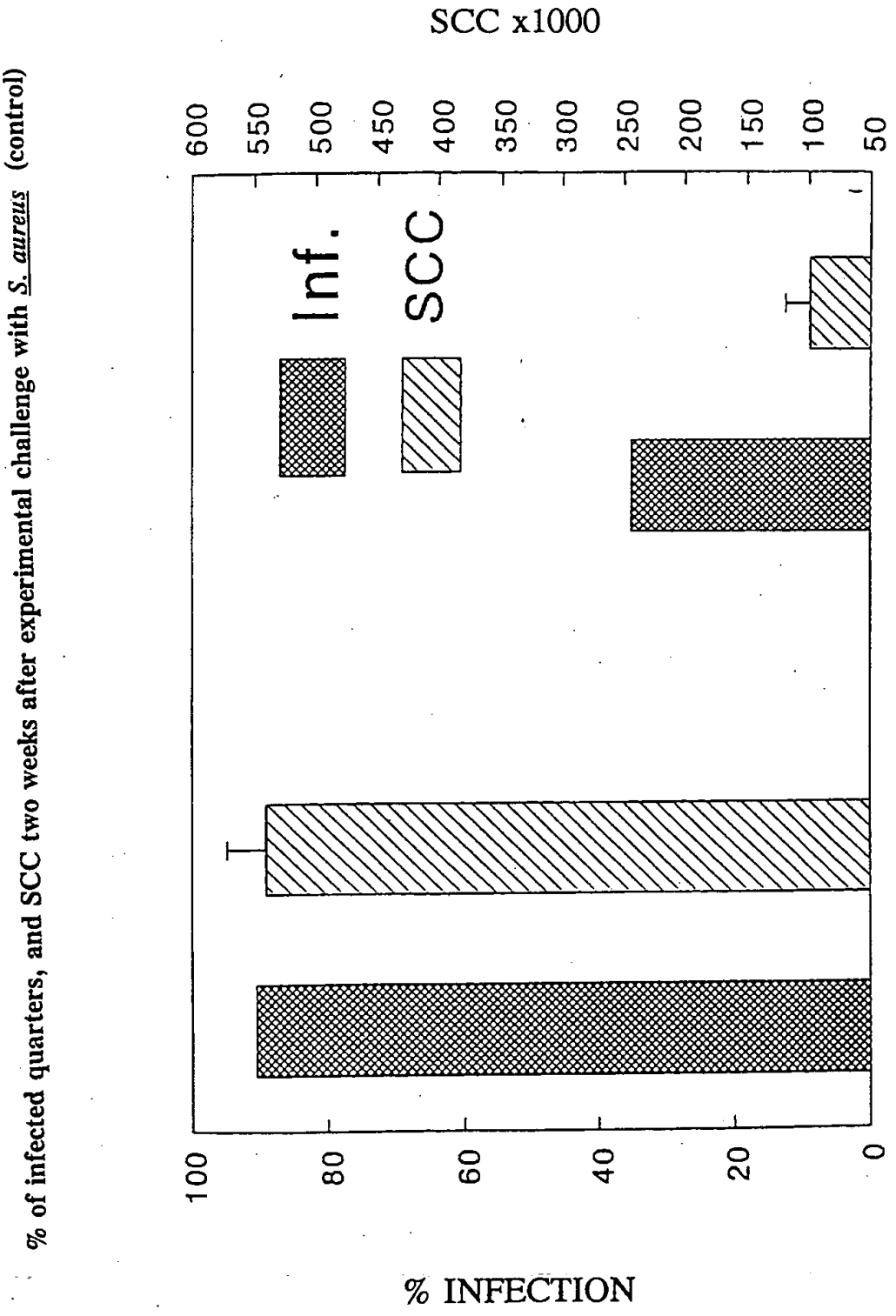


Fig 3



% of infected quarters, and SCC two weeks after experimental challenge with *S. aureus* (control)

Fig 4

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/IL 98/00627

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12R A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU 85929 82 A (COMMW SCIENT IND RES ORG) 20 January 1983 see abstract see page 2, line 22 - page 3, line 16 see page 4, line 9 see claims 1,3,6 ---	1-18
A	WATSON D L: "Staphylococcal mastitis vaccine 'letter;comment!." VACCINE, (1992) 10 (5) 359, XP002100866 see the whole document --- -/--	13-18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 April 1999	06/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/IL 98/00627

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	NICKERSON S C ET AL: "Effect of a Staphylococcus aureus bacterin on serum antibody, new infection, and mammary histology in nonlactating dairy cows" J DAIRY SCIENCE, vol. 76, 1993, pages 1290-1297, XP002100867 cited in the application see abstract see page 1290, column 2, line 9 - page 1291, column 2, line 11 see page 1291, column 2, line 21 - line 24	13-18

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 98/00627

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 17, 18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 17, 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte .onal Application No
PCT/IL 98/00627

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
AU 8592982 A	20-01-1983	NONE	